

CLAIMS

1. A method of generating storage-stable competent cells, said method comprising:
 - a) growing bacterial cells in culture medium at hyperosmotic salt concentration;
 - b) treating said cells to make them competent;
 - 5 c) contacting said cells with a solution comprising a reducing sugar or a non-reducing sugar, or both; and
 - d) drying the competent cells resulting from step (c) in the presence of a non-reducing sugar, such that storage-stable competent cells are generated.
2. The method of claim 1 wherein said salt is NaCl.
- 10 3. The method of claim 1 wherein said hyperosmotic salt concentration is 100 mM to 350 mM above isoosmotic.
4. The method of claim 1 wherein said hyperosmotic concentration of NaCl is 150 mM to 225 mM above isoosmotic.
5. The method of claim 1 wherein said hyperosmotic salt concentration is 200 mM
- 15 above isoosmotic.
6. The method of claim 1 wherein step (c) is performed either during or after step (b), but before step (d).
7. The method of claim 1 wherein said drying step is performed at a temperature above freezing.
- 20 8. The method of claim 1 wherein step (d) comprises drying said cells in the presence of a non-reducing sugar selected from the group consisting of trehalose, sucrose, α -methyl glucopyranoside, α -methyl galactopyranoside, and sorbitol.

9. The method of claim 1 wherein step (c) comprises contacting said cells with a non-reducing sugar selected from the group consisting of trehalose, sucrose, α -methyl glucopyranoside, α -methyl galactopyranoside, and sorbitol.
10. The method of claim 1 wherein step (c) comprises contacting said cells with a
5 reducing sugar selected from the group consisting of fructose, glucose (dextrose), maltose, lactose, glucopyranose, ribose and cellobiose.
11. The method of claim 9 wherein step (c) comprises contacting said cells with a non-reducing sugar selected from sorbitol and α -methyl glucopyranoside.
12. The method of claim 10 wherein step (c) comprises contacting said cells with a
10 reducing sugar, wherein said reducing sugar is fructose.
13. The method of claim 1 wherein step (c) comprises contacting said cells with fructose and a non-reducing sugar selected from sorbitol or α -methyl glucopyranoside.
14. The method of claim 10 wherein in step (c), or step (d) said reducing sugar and said non-reducing sugar is present at a total sugar concentration of 10-25% (w/v).
15. 15. The method of claim 1 wherein said cells are made competent by exposure to a
chemical agent.
16. The method of claim 15 wherein said chemical agent is selected from the group consisting of CaCl_2 , RbCl_2 , MnCl_2 , and hexamine cobalt chloride.
17. The method of claim 1 wherein said step of drying the competent cells is
20 performed under vacuum.
18. The method of claim 17 wherein said step of drying the competent cells is performed at a temperature above freezing.

19. The method of claim 1 step (a) comprises growing said bacterial cells to a final OD550 of 0.45 to 0.5.
20. The method of claim 1 wherein said bacterial cells are Gram negative cells.
21. The method of claim 1 wherein said culture medium comprises casein hydrolysate
5 and/or maltose.
22. The method of claim 21 wherein said casein hydrolysate is present in said culture medium at a concentration of 11-15 g/liter.
23. The method of claim 21 wherein said casein hydrolysate is present in said culture medium at a concentration of 11-12 g/liter, inclusive.
- 10 24. The method of claim 21 wherein said maltose is present in said culture medium at a concentration of 0.1-0.3 % (w/v).
25. The method of claim 21 wherein said maltose is present in said culture medium at a concentration of 0.2-0.3% (w/v), inclusive.
26. The method of claim 1 wherein said step of treating cells to make them competent
15 comprises contacting said cells with a defined solution comprising one or both of proline and threonine.
27. The method of claim 26 wherein said defined solution comprises proline, threonine or both at a concentration of 0.5-7.5 mg/ml.
28. The method of claim 26 wherein the concentration of proline, threonine or both in
20 said defined solution is from 2-4 mg/ml, inclusive.
29. The method of claim 1 wherein said competent cells can be stored at temperatures above -80°C for at least one month and maintain transformation efficiencies of at least 105 colonies/μg DNA.

30. The method of claim 1 wherein said competent cells can be stored at temperatures of -20°C or above for at least one month and maintain transformation efficiencies of at least 10⁵ colonies/μg DNA.

31. The method of claim 1 wherein said competent cells can be stored at temperatures of 0°C or above for at least one month and maintain transformation efficiencies of at least 10⁵ colonies/μg DNA.

32. The method of claim 1 wherein said competent cells can be stored at temperatures of 4°C or above for at least one month and maintain transformation efficiencies of at least 10⁵ colonies/μg DNA.

33. The method of claim 1 wherein said competent cells can be stored at temperatures of 15°C or above for at least one month and maintain transformation efficiencies of at least 10⁵ colonies/μg DNA.

34. The method of claim 1 wherein said competent cells can be stored at temperatures of 20°C or above for at least one month and maintain transformation efficiencies of at least 10⁵ colonies/μg DNA.

35. The method of claim 1 further comprising the step, during or after step (c), of limiting the exposure of said competent cells to oxygen.

36. The method of claim 35, comprising the step, after step (c), of storing said competent cells in a vacuum stoppered vial.

37. The method of claim 36, comprising the step of storing said vial in a sealed pouch.

38. The method of claim 35 wherein said limiting comprises drying and/or storing said competent cells in the presence of an oxygen scavenger.

39. The method of claim 1 further comprising the step, after step (c) of limiting exposure of said competent cells to moisture.

40. The method of claim 39 wherein the stopper in said stoppered vial is baked to remove moisture prior to use.

5 41. The method of claim 1 further comprising the step, during or after step (c), of limiting the exposure of said competent cells to light.

42. The method of claim 41 wherein said limiting comprises storing said competent cells in a vial that has reduced transmittance of light.

43. A preparation of storage stable competent cells prepared according to claim 1.

10 44. A kit comprising the preparation of claim 43.

45. The method of claim 1 wherein step (d) comprises drying the competent cells resulting from step (c) in the presence of a non-reducing sugar and gelatin, such that storage-stable competent cells are generated.

46. The method of claim 45 wherein said gelatin is present at 0.5 to 2.5%

15 47. The method of claim 45 wherein said gelatin is present at 0.8 to 1.2%.

48. A method of producing a transformed cell, said method comprising

a) obtaining cells generated according to the method of claim 1;

b) re-hydrating said cells;

c) contacting said cells with a nucleic acid vector; and

20 d) growing said cells, such that a transformed cell is produced.

49. A method of producing a recombinant polypeptide comprising:

a) obtaining cells generated according to the method of claim 1;

b) rehydrating said cells;

c) contacting the rehydrated cells with a nucleic acid encoding said recombinant polypeptide; and

d) growing said cells in a cell growth medium under conditions in which the cells produce said polypeptide.

5 50. The method of claim 48, in which cells which have taken up said nucleic acid are separated from cells which have not taken up said nucleic acids.

51. The method of claim 49, wherein said recombinant polypeptide is isolated from said cells.